Effect of Conspecifics and Crayfish on Zebra Mussel Defense Behavior

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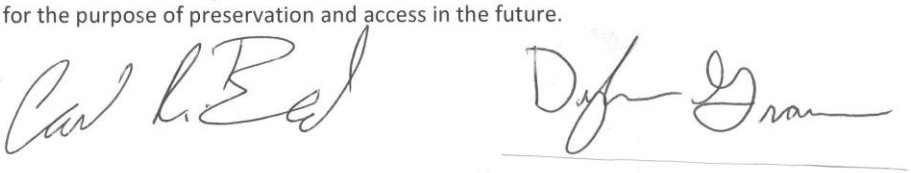
Abstract:

Zebra mussels (Dreissena polymorpha Pallas, 1769) have profoundly negative effects on both Great Lakes aquatic ecosystems through the decline of native bivalve species and on economic activities, costing industry upwards of $150,000,000 yearly. Successful control of these invasive species would be beneficial for the Great Lakes region as a whole. Recently, studies have shown that zebra mussels respond to both injured mussel conspecifics and to predator kairomones, but there has been no conclusive evidence to suggest that either has more of an effect than does the other. In a laboratory setting, we tested this question using four treatments: a control tank, a tank containing crushed mussel conspecifics, a tank containing crayfish that had eaten mussels, and a tank containing crayfish exclusively. Testing the distance that mussels traveled as well as the aggregation rates of mussels in each treatment tank, we determined that mussels move significantly farther when exposed to crushed mussel conspecifics than in the absence of any treatment. While not significant, trends show that crushed conspecifics have the greatest effect on mussel clumping rates as well. This signifies that the presence of injured mussels triggers greater behavioral changes in zebra mussel populations than does the presence of predator kairomones. Further research regarding this subject may help determine effective, natural means by which to limit invasive mussel populations.



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Introduction:

Zebra mussels (*Dreissena polymorpha* Pallas, 1769) are invasive bivalve species native to the Ponto-Caspian range whose introduction into many Western European and North American habitats causes major problems for native mollusk species and wildlife management efforts in the invaded areas (Lewandowski 2001). Using byssal threads, zebra mussels attach to a variety of sources, specifically boat hulls, which allows for the movement of mussels across long distances and explains their arrival into the Great Lakes region by 1985-1986. Mussels can reproduce early (as soon as water reaches approximately 12^0C, or generally in May) and often, with a female mussel capable of producing several hundred thousand eggs per season (Borcherding 1991). Mussels grow rapidly and can live up to three years in the Great Lakes region (Snyder et al. 1990). In addition, their survival rate is quite high, leading to an abundance of individuals. The mussels can withstand large variations in light, hydrostatic pressure and temperature, allowing them to survive in a wide array of habitats. Further, since they are filter feeders, mussels can consume many types of algae, phytoplankton, and detritus in the water. The explosion in North American mussel population may be partly due to a lack of bacteria or disease that limits population potential; while there are plenty of mussel predators, there are not enough to control population.

Zebra mussels are a major problem for several reasons. They are often better competitors than native North American mussel and clam species, creating decreased native fitness and population loss. Zebra mussels may compete by filtering food used by native species (due to niche overlap) or may attach via byssal threads to clams themselves, causing a decline in the fitness of native individuals. Habitat loss is another issue as zebra mussels invade space and

dominate the shoreline ecosystems. Because there are several endangered mollusk species in the Great Lakes region, this poses serious extinction risks. Further, the increasing zebra mussel numbers pose problems for industry and human interactions as mussels attach to machinery and cause functionality issues, resulting in over $150,000,000 in indirect and direct damages (O'Neill 1997).

In the past, programs implementing a variety of different control mechanisms ended with mixed results. Researchers studying the effects of temperature on zebra mussel mortality found positive mortality rates at temperatures over 90^0F and determined that certain paints containing tributylin polymers or chemicals like chlorine have worked in limiting zebra mussel populations (ENSR International 2005). These methods, however, are ineffective overall as high temperatures and toxic chemicals and paints are detrimental to a wide variety of aquatic species and the overall health of water sources. Manual or mechanical scraping of zebra mussels on substrate is costly and inefficient. Thus, studies determining new and safer or natural methods could be helpful in controlling populations.

Recently, researchers conducted several studies considering zebra mussel defense mechanisms, which, if found, we can manipulate to alter zebra mussel prevalence. Mussels have several defenses to resist predation, including the development of various shell shapes and sizes to resist certain predators, aggregating into groups, and the formation of stronger substrate attachments (Diaz 2010). Researchers found that zebra mussels respond to kairomones or conspecifics in the water as a defense mechanism. The presence of these kairomones, or chemical signals released by the predator, may cause a change in movement patterns as well as in mussel clumping and aggregation (Ferrari et al. 2010). Studies show that in response to predatory cues, mussels will decrease physiological processes to lower metabolite emissions in

an attempt to hide their locations (Czarnoleski et al. 2010). Further, the introduction of alarm substances from conspecifics in lab conditions can cause an inhibition in movement (Kodek et al. 2009). Studies also show that we should see greater clumping in the presence of conspecifics and predator kairomones (Kodek 2000). It is advantageous to resist movement and attach to a substrate, as attached mussels are more difficult to dislodge and consume than unattached mussels; therefore, individuals that can attach to substrate tend to have higher fitness since mussels do not escape predation well via movement (Toomey et al. 2002).

With knowledge of zebra mussel tendency of substrate attachment and decrease movement in the presence of conspecifics and predator chemicals, we can start to formulate more efficient and effective management plans. Efforts to introduce zebra mussel conspecifics or predator kairomones into Great Lakes water bodies could result in greater zebra mussel clumping, allowing for more effective removal methods, such as scraping. The centralization of zebra mussel species occurring due to aggregation would allow for more targeted management actions, as there will be fewer lone, unattached individuals. This may cause problems for predation, however, as clumped individuals tend to have a defensive advantage. Introducing predator species with weaker kairomones or those with better predation techniques that result in a smaller release of mussel conspecifics would help to minimize clumping, thus allowing predators to feed effectively on mussel individuals.

Therefore, the study of predator-prey interactions in Great Lakes region freshwater areas can help determine the most successful means of controlling invasive mussel species. There are currently competing theories as to the chemicals and hormones that have the greater effect on zebra mussel behavior, with split decisions on whether a zebra mussel's response to the presence of a predator is due more to the release of predator kairomones or by the conspecifics released.

Using a strong freshwater predator, the northern crayfish (*Orconectes virilis*) that picks up and detaches mussels with their claws and breaks open shells with mandibles, we want to determine whether predator kairomones or mussel conspecifics have a greater effect on zebra mussel movement and aggregation. Because mussels tend to move less and clump together when disturbed, we predict to see lower total and average mussel movement with higher clumping rates when conspecifics or predator kairomones are present. Further, we predict to see an aggregate effect in cases where we exposed mussels to both conspecifics and kairomones, as both hormones seem to have an effect on mussel behavior.

Methods:

To determine zebra mussel movement and clumping behaviors in response to conspecifics and predators, we set up four aquariums, each containing 30 zebra mussels, and one of the following treatments: crayfish, crayfish with recent zebra mussel consumption, crushed zebra mussel conspecifics (15 mL), or the control (30 zebra mussels, no conspecifics or crayfish). Ten trials, each occurring over a 24-hour period, were completed for each treatment. The four aquariums (dimensions: 49 cm X 25 cm X 29 cm) each contained an approximate depth of 2-5 cm sand and 11 L of Douglas Lake water. This water had a temperature of 23^0C, pH of 8.55, and an EDTA hardness of 117.6 $CaCO_3$/L, which were constant across the tanks. We used three air pumps (brands: Silent Giant, Second nature Challenger II, and Elite 800) to maintain aeration in each tank. The tanks experienced similar natural light conditions. We divided each tank into two containment areas with ¼ of the tank for the treatments and the remaining ¾ of the tank for the zebra mussel tests (figure 1). This ensured the crayfish could not attack or move any zebra mussels, altering results in any way. We used zebra mussels 7-12 mm in length collected from Douglas Lake shoreline areas (Cheboygan Co., MI). In each tank, we placed ten zebra

mussels, each marked with a different nail polish color to differentiate when collecting data. On a corresponding petri dish (diameter: 8.1 cm) we marked the same ten colors at 1 inch intervals. We used this dish to determine the starting point of each zebra mussel so that we could track its movement over the 24-hour treatment intervals. To test the effect of conspecifics and predators on aggregation and clumping, we placed 20 unmarked zebra mussels into each treatment tank at random positions. We measured aggregation as any clump containing two or more zebra mussels attached via their byssal threads; we recorded the number of total clumps per treatment and the number of zebra mussels in each clump. We placed two crayfish individuals each in the crayfish with zebra mussel and crayfish alone treatment tanks. For the crayfish with zebra mussel diets, we set up a tank with the crayfish and a number of zebra mussels and allowed them to feed overnight while we ran trails from the previous day. We caught crayfish from Burt Lake, Cheyboygan Co., MI, which varied in carapace length between 21 and 48 mm. To ensure that we did not skew results in any way, we replaced the water between each trial and used new crayfish for each trial. After compiling data, we totaled and averaged the distance travelled for all marked individuals of each treatment. We also compiled the total and average number of clusters for each treatment. We conducted a two-way ANOVA statistical test to determine whether there were statistically significant differences between both zebra mussel clumping and movement in each of the four treatments. Further, we used Tukey's post hoc tests to determine

whether any of the mean distances travelled were significantly different from each other.

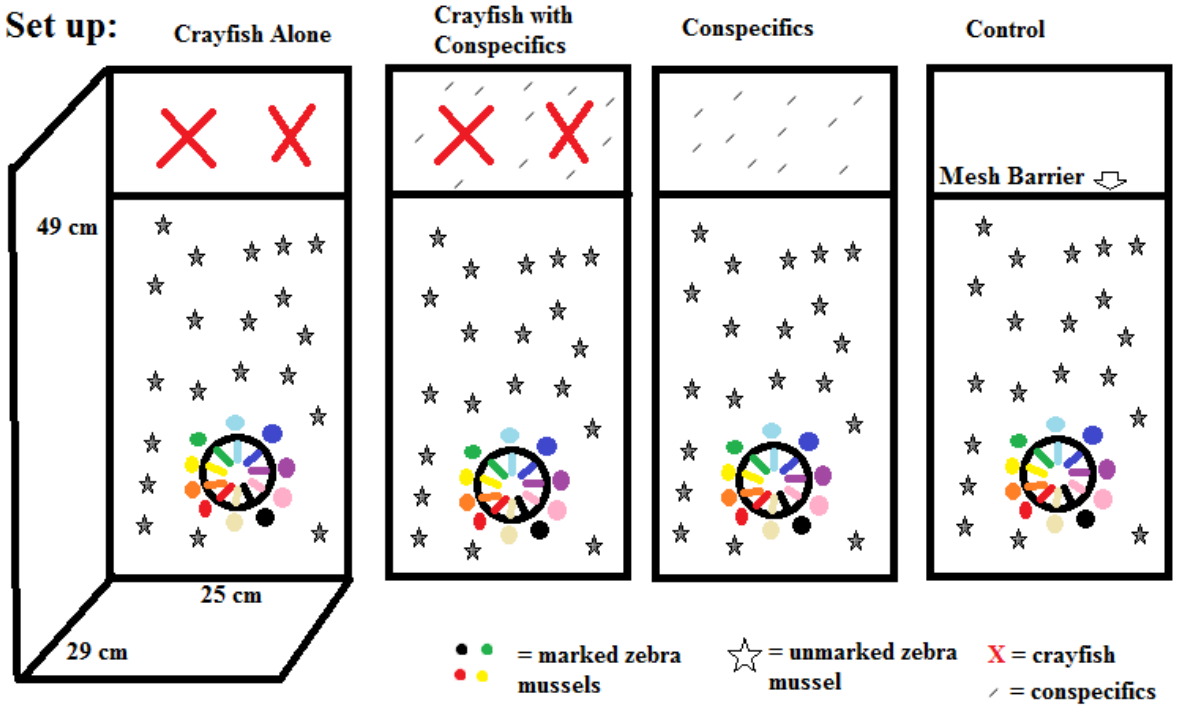

Figure 1: A Diagram of the Experimental Set-up

Results:

Zebra mussel movement varied substantially between treatments. The mean distance travelled for zebra mussels was 7.84 cm in the control tank, 10.36 cm in the crushed conspecifics tank, 9.82 cm in the crayfish tank, and 11.48 cm in the tank with zebra mussel fed crayfish (table 1). Figure 1 shows the mean distances travelled with 95% confidence intervals included. From the two-way ANOVA test, we found that the treatments containing crushed conspecifics or mussel-fed crayfish were significantly different from the control. From this data, we can see that distance traveled increased by 46.4% when exposed to crayfish that had consumed conspecifics, 32.14% in the presence of crushed conspecifics, and 25.2% in the presence of crayfish exclusively. The p-value for conspecifics was 0.013. The p-value for treatments containing

crayfish was 0.103 (table 2). The mean number of zebra mussel clumps over the ten trials was 3.0 in the control tank, 3.2 in the crushed conspecifics tank, 2.9 in the crayfish tank, and 4.in the tank containing crayfish that had eaten mussel conspecifics. The average number of zebra mussels aggregated in any given treatment was 1.4 in the control tank, 1.5 in the crushed conspecifics tank, 1.4 in the crayfish tank, and 2.2 in the tank containing crayfish that had eaten zebra mussels (table 1). Figure 2 shows the number of clumps per treatment and figure 3 shows the average number of mussels per clump in each treatment. The amount of aggregation that each treatment caused was not significantly different, however, as the one-way ANOVA test reported a p-value of 0.525 between the number of aggregations across treatments and a p-value of 0.452 between the numbers of clumped zebra mussels across treatments (table 3).

Discussion:

Results indicate that the presence of crushed zebra mussel conspecifics and crayfish kairomones has an effect on zebra mussel behavior. When exposed to crushed mussel conspecifics or crayfish that had eaten mussels, zebra mussels moved significantly further, on average. The increased movement may be defensive as zebra mussels try to move away from the predator, thus avoiding predation. While the treatments with crayfish were not significantly different from the control, we can still say that zebra mussels are moving further due to predation, since the crushed mussel conspecifics simulate injured zebra mussels. The response to crushed conspecifics indicates that injured mussels affect zebra mussels more than the predator's kairomones. However, the kairomones seem to have an additive effect on zebra mussel behavior, as figure 1 indicates. Further, while the difference in distance traveled is not statistically significant, trends show that zebra mussel movement is higher when exposed to crayfish. The data does not hold with our hypothesis, as we had previously estimated that

average mussel movement would decrease, in part due to an increased rate of clumping. The results directly oppose a previous study determining the factors affecting zebra mussel movement, as the researchers in that case concluded that injured mussel conspecifics caused the zebra mussels in their trials to move less (Toomey et al. 2002). Our results may be due to individuals seeking substrates on which to attach and thus had to move further, since the only substrates in our tank were the walls and the mesh divider between the mussel and the conspecifics or crayfish. We found many individuals on both the divider and on the walls, which seems to support the aforementioned hypothesis.

The presence of crushed conspecifics and kairomones did not significantly change zebra mussel clumping behavior. While we cannot say that results are significant, several trends exist, indicating that we may have found stronger results if we had conducted more or longer lasting trials. The trends show that zebra mussels attach to substrate most frequently when crushed conspecifics are present. Attachment decreased when crayfish were present, indicating that the presence of predator kairomones has little effect on the attachment and aggregation rates of zebra mussel individuals. Attachment is a defense mechanism, as studies show that increased attachment decreases the likelihood that predators successfully break open and consume the mussels (Côte and Jelnikar 1999). There are many potential reasons why results were not statistically significant. It is possible that *O. virilis* does not produce enough kairomones for mussels to detect; further, it is possible that we did not use enough conspecifics in the tank for mussels to respond with clumping behaviors.

Wildlife management officials and researchers can potentially use this information to determine effective control and eradication methods for invasive mussel species in the Great Lakes region and other North American waterways. Since mussels respond better to injured

mussel conspecifics than to predator kairomones, the introduction of crushed mussel conspecifics into water bodies could be a large step in the right direction, as results show that mussels will increase movement and possibly increase aggregation rates. Both of these actions would be beneficial for a number of reasons. The aggregation of mussels would lead to easier mussel removal, as clumps of mussels and mussels on rocks and other substrates are easier to remove than are isolated individuals. The introduction of mussel conspecifics in one area of a lake may cause mussels to move to other areas and aggregate, which could deplete mussel populations in deeper or hard to reach areas of the water. Although adding mussel predators seems beneficial since the combination of predators and conspecifics is effective in controlling zebra mussel behavior and studies show that they are successful, it would be harder to introduce predators into a waterway and may result in native species loss since there are no predators specifically adapted to zebra mussel consumption (Ermgassen and Aldridge 2011). While the results do not provide any simple eradication methods, they certainly provide ways by which we could make the manual removal of mussels easier. This in turn would take pressure off native bivalve species and ultimately preserve the biodiversity of the Great Lakes region. Further study into native pathogens, viruses, or bacteria would also be helpful in determining effective means by which to control the invasive populations, as these biological control agents may have more direct and efficient effects on mussel eradication.

Appendix:

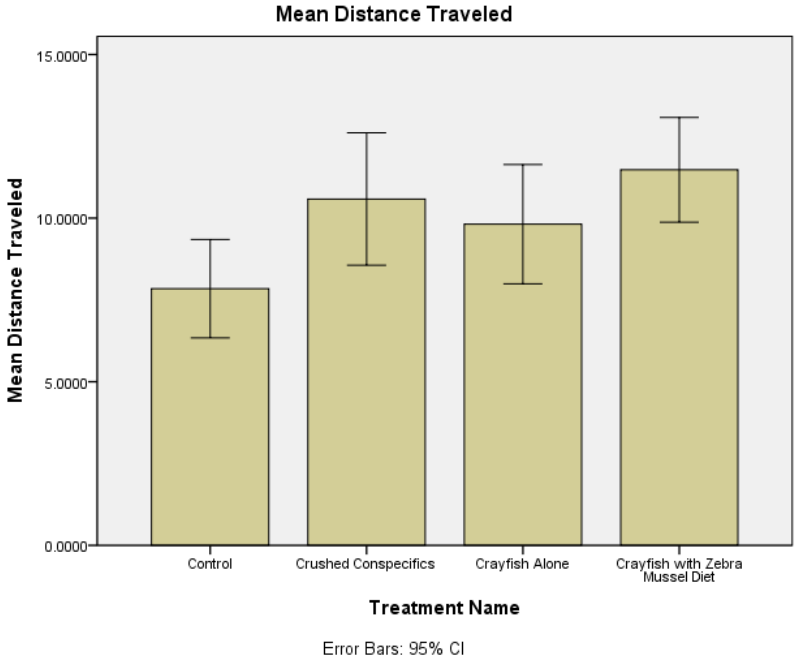

Figure 1: Zebra mussels exposed to crushed conspecifics move more often than when exposed to no treatment or crayfish exclusively.

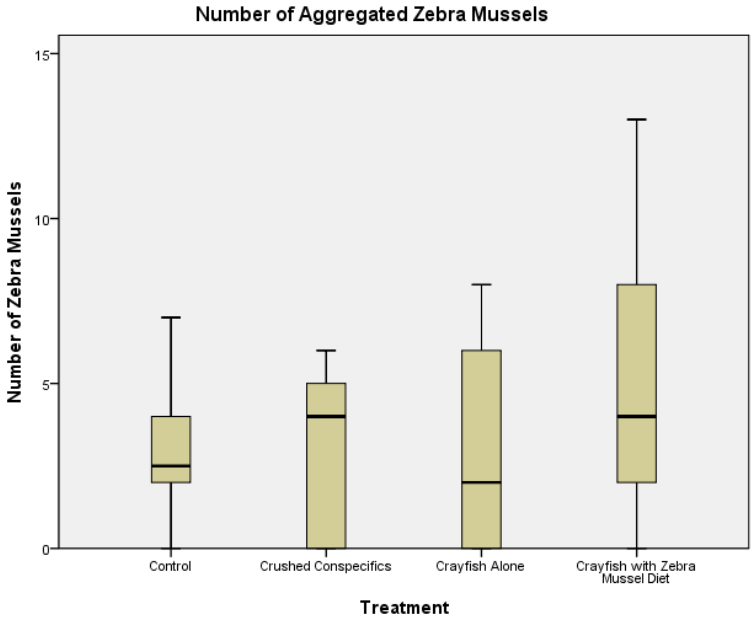

Figure 2: While crushed conspecifics do cause a slightly higher incidence rate of clumping amongst zebra mussels, the data is not significant.

Treatment	Mean Distance Traveled by Zebra Mussels	Mean Number of Clumps Present	Mean number of Zebra Mussels aggregated
Control	7.84 (7.54)	3 (2.31)	1.4 (1.07)
Crushed Conspecifics	10.36 (9.95)	3.2 (2.49)	1.5 (1.18)
Crayfish Alone	9.82 (9.04)	2.9 (3.14)	1.4 (1.51)
Crayfish with Zebra Mussel Diet	11.48 (8.02)	4.9 (4.25)	2.2 (1.75)

Table 1: The average distance that zebra mussels traveled, the mean number of aggregated zebra mussels, and the mean number of clumps was highest when exposed to crayfish that had consumed zebra mussels, indicating that both crushed conspecifics and crayfish kairomones have some effect on zebra mussel defense behavior.

Tests of Between-Subjects Effects

Dependent Variable:Distance Traveled

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	710.164[a]	3	236.721	3.151	.025
Intercept	38438.544	1	38438.544	511.666	.000
Conspecifics	471.978	1	471.978	6.283	.013
Crayfish	200.392	1	200.392	2.667	.103
Conspecifics * Crayfish	28.553	1	28.553	.380	.538
Error	28997.949	386	75.124		
Total	68110.420	390			
Corrected Total	29708.112	389			

a. R Squared = .024 (Adjusted R Squared = .016)

Table 2: The distance that zebra mussels traveled in tanks containing crushed mussel conspecifics was significantly higher than in the control treatment. The distanced traveled in tanks containing crayfish, however, was not statistically significant.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
# of clumps	Between Groups	4.475	3	1.492	.757	.525
	Within Groups	70.900	36	1.969		
	Total	75.375	39			
Total Clustered	Between Groups	26.600	3	8.867	.898	.452
	Within Groups	355.400	36	9.872		
	Total	382.000	39			

Table 3: There is no significant difference between the number of mussels clumped or the number of total clumps between any groups, signifying that while trends exist, there is no significant clumping data.

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